Investigation of lymphatic network and cancer metastasis

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論 文 内 容 の 要 旨

The objectives of this thesis were to clarify the distributions of lymph nodes (LNs) and lymph vessels (LVs), the communication between lymphatics and the surrounding veins, and the relationship between cancer metastasis and the lymphatic system in MXH10/Mo/lpr, a lympho-proliferative in-bred mouse, to extrapolate the findings to develop potential new clinical treatments.

In Chapter 1, overviews of cancer metastasis, the lymphatic system and the relationship between them are outlined. An extensive literature review strongly indicated that the lymphatic system facilitates cancer cell dissemination throughout the body, acting in concert with the venous system. It is clear that preclinical models of LN metastasis are indispensable to aid the understanding of the mechanisms involved in tumor cell trafficking, which are fundamental for developing new techniques to diagnose and treat LN metastasis.

However, the identification of LNs and LVs in mice is challenging with conventional imaging modalities, since the LN diameter in normal mice is 1–2 mm. In this thesis, a preclinical model of LN metastasis has been used involving MXH10/Mo-lpr/lpr (MXH10/Mo/lpr) inbred mice, which develop systemic swelling of LNs up to 10 mm in diameter.

In Chapter 2, the aim was to identify LNs and LVs in MXH10/Mo/lpr mice and establish one of the peripheral inter-LN routes that can induce regional lymphatic cancer metastasis. Twenty-two different LNs were found distributed in the peripheral, thoracic and abdominal regions, and 4 peripheral inter-LN vessels were identified, from the subiliac LN (SiLN) to the proper axillary LN (PALN), the parotid LN to the caudal deep cervical LN, and the popliteal LN to both the sciatic LN and the SiLN. Moreover, peripheral regional metastasis was induced by inoculating FM3A/Luc mouse breast cancer cells into the SiLN. This chapter unveils the anatomy of murine lymphatics to give new insights into the investigation of inter-LN metastasis of cancer, especially the mechanisms involved in the trafficking of cancer cells in inter-LN vessels. The lymphatic anatomy data will be useful in the quest for deepening our understanding of cancer cell trafficking and the mechanisms involved in cancer metastasis. The results also show, for the first time, that the MXH10/Mo/lpr mouse strain is an 'investigator-friendly' and reliable model of peripheral inter-LN cancer metastasis.

In Chapter 3, the communication pathways between the lymphatic and venous systems are clarified, with a focus on the anatomy of these communication routes in the axillary and subiliac regions. The communication pathways between the lymphatic and venous systems in the axillary and subiliac regions of mice were unequivocally identified. The efferent LVs of the PALN were demonstrated to communicate with the subclavian vein. Furthermore, it was shown that the thoracoepigastric vein (TV), which connects the subclavian vein and inferior vena cava, runs adjacent to the SiLN and PALN, and receives venous blood from these LNs routed through small branches. The direction of blood flow in the TV occurred in two directions in the intermediate region between the PALN and SiLN; one to the subclavian vein, the other to the inferior vena cava. This research reveals the anatomy of the communication between the lymphatic and venous systems in the axillary and subiliac regions of the mouse, and provides further lymphatic-venous anatomy data relevant to the investigation of the trafficking routes of cancer cells in preclinical mouse models. The bi-directional flow of blood in the TV between the PALN and SiLN gives insight into tumor cell trafficking from regional areas to the whole body. It is proposed that the final form of LN metastasis should be recognized as “LN-mediated hematogenous metastasis” based on lympho-venous communication.

In Chapter 4, the surgical and non-surgical outcomes of an implanted LN tumor were explored, with a focus on regional cancer and distant metastasis. The tumor-bearing SiLN was resected to simulate clinical dissection of the sentinel lymph node (SLN). It was found that resection of a tumor-bearing SiLN enhanced lung metastasis in the mouse model. Bioluminescence imaging revealed that metastatic tumor cells in the down-stream LN continued to grow after the resection of the up-stream tumor-bearing SiLN, and that the probability of metastasis to the lungs was increased when the interval between SiLN inoculation and resection was reduced. Furthermore, histological analysis demonstrated that latent cancer cells in the lungs were stimulated to grow after resection of the SiLN. Fluorescence imaging indicated that the route of tumor cell dissemination from the SiLN to the lungs was via the venous system enveloping the SiLN. This part of the thesis confirmed two trafficking routes in the SiLN region: one towards the PALN via the LV, which is related to PALN lymphogenous metastasis, and the other towards the venous system via the TV, which is related to hematogenous metastasis to distant sites. The resection of a SiLN inoculated with tumor cells led to an accelerated growth of metastatic tumor cells in the lungs and ipsilateral PALN. This result is a timely reminder of the clinical risk of iatrogenic induction of regional and distant cancer metastases. This phenomenon provides new insights into the concept of “LN-mediated hematogenous metastasis” and is the starting point for tracing the activation process of distant dormant cancer cells.

In Chapter 5, it is concluded that the lymphatics can facilitate cancer cell dissemination, and that this is fundamental to the occurrence of cancer metastasis. Presently, two contrasting models are used to explain the formation of distant organ metastases: a lymphatic-independent hematogenous model and a lymphatic-dependent sequential model. The most crucial issue to resolve is whether metastatic cells come to a halt in LNs or continue to disseminate throughout the body by usurping either LN vascular vessels or efferent LVs to colonize distant organs via the blood circulation. To date, the detailed mechanisms involved in this important process remain a mystery. However, there is now little doubt that the interplay between tumors and the lymphatic system represents the main route used by solid cancers to spread.

Based on a study of MXH10/Mo/lpr mice, this dissertation shows the topography of 22 LNs and 4 peripheral lymphatic drainage routes, which can be used to explore peripheral lymphatic cancer metastasis in a

preclinical setting. In addition, the thesis has clarified the communications between the lymphatic and venous systems in the axillary and subiliac regions, and revealed that the LV running from the SiLN to the PALN is capable of draining tumor cells. Moreover, the research in this thesis has also shown that lung metastatic foci could be rapidly activated following resection of a tumor-bearing SiLN. It is anticipated that this mouse model will be useful for studying LV imaging, lymphatic trafficking kinetics and the mechanisms of tumor-lymphatic system interplay during both regional and distant metastasis, with the aim of translating this basic research into enhanced clinical diagnosis and novel drug delivery systems.

論文審査結果の要旨

多くのがん種ではリンパ節転移が確認されているが，リンパネットワークとがん転移との関連性は十分に解明されていない．その理由としてリンパ節転移をモデル化するための疾患モデルマウスの欠如が上げられる．本論文は，リンパ節腫脹マウス MXH10/Mo-*lpr*/*lpr* (MXH10/Mo/lpr) を使用し，リンパ節の解剖学的な位置，リンパ節を介するリンパ系と静脈系との交通，リンパ節郭清と遠隔転移との関係を示すことで，リンパネットワークとがん転移との関係を明らかにすることを目的にしており，全編５章からなる．

第１章は緒論であり，本研究の背景，目的および構成を述べている．

第２章では，MXH10/Mo/lpr マウスにおいて２２種類のリンパ節の解剖学的位置と名称を確定し，かつ４種類の末梢リンパ経路を同定している．マウス乳がん細胞を MXH10/Mo/lpr マウスの腸骨下リンパ節に移植することで内側腋窩リンパ節に転移が誘導されることが示され，MXH10/Mo/lpr マウスを転移モデルマウスとして利用できることを世界で初めて実証している．

第３章では，がん転移に関わるリンパネットワークと静脈系との交通を精査するために，腋窩部と腸骨下部におけるリンパネットワークと静脈系を調べた．胸腹壁静脈が腸骨下リンパ節と内側腋窩リンパ節を介して鎖骨下静脈と下大静脈に連結することを明らかにし，また，腸骨下リンパ節と内側腋窩リンパ節の中間付近では胸腹壁静脈に他の静脈が連結することで，胸腹壁静脈の血流の流れがこの静脈連結部で腸骨下リンパ節方向と内側腋窩リンパ節の方向に二分されることが示された．また，腸骨下リンパ節に蛍光色素を注入すると輸入リンパ管と胸腹壁静脈に蛍光色素が流れ出ることから，リンパ節転移と血行性転移は明確に区別できるものではなく，リンパ節を起点として血行性転移に転じる転移経路であるというリンパ節介在性血行転移の概念を世界で初めて提唱した．

第４章では，リンパ節郭清後の比較的早期に遠隔転移が生ずるという臨床事象の機序を明らかにするために，腫瘍細胞が移植された腸骨下リンパ節を切除することで生じる肺転移の活性化を評価した．肺での遠隔転移は腫瘍移植リンパ節の切除によって活性化されることが示され，臨床におけるリンパ節郭清後の遠隔転移病原の活性化モデルを示した．

第６章は結論であり，各章の成果をまとめている．

以上要するに本論文は，リンパネットワークとがん転移の関係を明らかにするために，疾患モデルマウスにおけるリンパ節の解剖学的な位置，名称の定義，末梢リンパ経路の同定，リンパ節を介したリンパ系と静脈系の交通の解明，リンパ節介在性血行転移の概念の提唱，転移リンパ節郭清にともなう遠隔転移の活性化の可能性を明らかにし，リンパ系とがん転移に関する新たな知見を与えたものであ，医工学や臨床科学の発展に寄与するところが少なくない．

よって，本論文は博士（医工）の学位論文として合格と認める．